$CD4^+$ T Cell Responses to Interleukin-2 Administration in HIV-Infected Patients Are Directly Related to the Baseline Level of Immune Activation

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Background. Intermittent interleukin (IL)–2 administration to human immunodeficiency virus (HIV)–infected patients leads to $CD4^+$ T cell expansions. The factors potentially affecting these expansions were investigated in the present study.

Methods. A matched (for baseline $CD4^+$ T cell count) case-control study was designed. Nonresponders (NRs) were defined as patients with a $\leqslant$10% increase in $CD4^+$ T cell count 2 months after the third IL-2 cycle (week 24), compared with that at baseline (week 0). Control subjects experienced a $\geqslant$50% increase in $CD4^+$ T cell count at week 24. Immunophenotype, Ki67 and forkhead box protein P3 (FoxP3) expression, and T cell receptor excision circle (TREC) measurements in T cells were evaluated at weeks 0 and 24 in both groups.

Results. Control subjects and NRs did not differ significantly at baseline in age, viral load, $CD4^+$ T cell count, nadir $CD4^+$ T cell count, or $CD8^+$ T cell count. At week 0, NRs had lower TREC levels per 1×10^6 T cells and higher levels of T cell proliferation and activation than did control subjects. At week 24, both groups experienced decreases in T cell proliferation and increases in CD25 and FoxP3 expression on $CD4^+$ T cells, with TREC levels per 1×10^6 $CD4^+$ T cells decreasing significantly only in control subjects.

Conclusions. Increased immune activation can adversely affect $CD4^+$ T cell expansions after IL-2 administration. Despite the lack of expansion, other evidence of IL-2–induced biological activity was observed.

Phase 2 studies have demonstrated that intermittent interleukin (IL)–2 administration to HIV-infected patients leads to significant $CD4^+$ T cell expansions in most treated patients with baseline $CD4^+$ T cell counts >200 cells/μL [1–4]. The IL-2–induced $CD4^+$ T cell count increases are predominantly the result of peripheral T cell expansion and are directly associated with decreases in T cell turnover [5, 6]. Phenotypically, the expanded $CD4^+$ T cells have a naive or central memory phenotype, express CD25, are long-lived, and express high levels of forkhead box protein P3 (FoxP3) [4, 7, 8]. The clinical significance of IL-2–induced $CD4^+$ T cell expansions is currently under investigation in 2 phase 3 studies [9].

The factors that may affect the degree of $CD4^+$ T cell responses to IL-2 are not clear. Low nadir $CD4^+$ T cell count has been found to be associated with poor $CD4^+$ T cell responses to IL-2 [10]. Additionally, it is unknown whether and to what degree the T cell pools of patients without $CD4^+$ T cell responses are affected by IL-2 immunotherapy.

In the present study, we sought to identify specific characteristics in the T cell pools of patients with poor $CD4^+$ T cell responses after IL-2 immunotherapy in combination with antiretroviral therapy (ART). It is plausible that lack or dysfunction of a specific subset

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of $CD4^+$ T cells—naive, central memory, or CD25 expressing—could negatively affect the $CD4^+$ T cell responses to IL-2. It is also possible that lack of $CD4^+$ T cell expansion by IL-2 could be related to such parameters as high propensity to apoptosis and high levels of immune activation, imposing an unfavorable balance between proliferation and cell death after IL-2 administration. To address this question, we identified 2 cohorts of patients distinguished by their responsiveness to IL-2 and sought baseline characteristics of their immune system that might predict responsiveness.

METHODS

Study design. Study participants were selected from a pool of 95 individuals who had participated in open-label National Institute of Allergy and Infectious Diseases–sponsored and institutional review board–approved studies using IL-2 (intravenously or subcutaneously) in combination with ART. Only studies with available stored cryopreserved specimens were screened. All participants had $CD4^+$ T cell counts >200 cells/μL at baseline and were receiving ART. None had received IL-2 previously.

These studies used an intermittent regimen of 5-day IL-2 (aldesleukin; Proleukin; Chiron) cycles administered every 8 weeks. IL-2 for intravenous administration was diluted in 5% aqueous dextrose solution containing 0.1% albumin and was given by continuous 24-h intravenous infusion with a compact portable pump. The starting dose of IL-2 ranged from 12 to 18 million IUs (MIUs) a day. This dose was decreased in increments of 1.5 to 6.0 MIUs as needed, to manage clinical or laboratory evidence of toxicity.

IL-2 administered subcutaneously was diluted in sterile water and injected subcutaneously at rotating sites. Administration of IL-2 was initiated at 7.5 MIUs twice a day subcutaneously for 5 consecutive days every 8 weeks. To manage toxicity, the IL-2 dose was decreased in increments of 1.5 MIUs/dose. Thereafter, dosage could be adjusted stepwise in either direction but was not to exceed the originally assigned dose or to decrease to <1.5 MIUs/dose.

A matched case-control study was designed. HIV-infected patients from this cohort with a $\leqslant$10% increase in $CD4^+$ T cell count (nonresponders [NRs]) were selected and matched for baseline (week 0, before IL-2 immunotherapy) $CD4^+$ T cell count with contemporaneous study participants (control subjects) who experienced a $\geqslant$50% increase in $CD4^+$ T cell count at the same time point. Response was determined based on $CD4^+$ T cell values obtained 2 months after completing the third cycle of IL-2 (week 24).

Baseline $CD4^+$ T cell counts represented the mean of the 3 values obtained before the initial administration of IL-2. Control subjects and NRs were additionally matched on the basis of their antiretroviral regimen (nucleoside analogues vs. highly active ART [HAART]) as well as the route of IL-2 administration (intravenous vs. subcutaneous). Patients who had initially received low doses of IL-2 (1.5 MIUs subcutaneously twice a day) due to the study design [1] were excluded from the selection process. These participants had consistently low $CD4^+$ T cell responses, which improved after transition to a higher-dose regimen. All participating patients had provided written informed consent.

Flow cytometry. Immunophenotypic analysis was performed on cryopreserved peripheral blood mononuclear cells from all study participants by use of 4-color immunofluorescence as described elsewhere [6]. The following monoclonal antibodies were used for staining: CD25 phycoerythrin (PE) (clone 2A3), CD38 PE (clone HB7), CD4 peridinin chlorophyll-a protein (clone SK3), CD3 allophycocyanin (APC), or fluoresceine isothiocyanate (FITC) (clone SK7), CD27 FITC (clone M-T271), HLA-DR FITC (clone L243), programmed death (PD)–1 PE (clone MIH4), and CD45RO PE or APC (clone UCHL-1) from BD Immunocytometry Systems and Pharmingen and forkhead box protein P3 (FoxP3) PE (clone 206D) from BioLegend. To study naive and memory phenotypes, subsets were defined by surface staining with CD3, CD4, CD45RO, and CD27 antibodies as described elsewhere [11]. Naive cells were defined as $CD45RO^-CD27^+$, central memory as $CD45RO^+CD27^+$, effector memory as $CD45RO^+CD27^-$, and effectors as $CD45RO^-CD27^-$. Intracellular staining for the nuclear antigen Ki67 and the cytoplasmic protein Bcl-2 was performed using Ki67 PE (clone B56) and Bcl-2 FITC (clone Bcl-2/100) from BD Immunocytometry Systems and Pharmingen following the protocols recommended by the manufacturer.

Samples were collected on a FACSCalibur (BD Immunocytometry Systems and Pharmingen) using CellQuest software. Approximately 1.0×10^5–1.2×10^5 total events with a minimum of 5000 events in the $CD4^+$ or $CD8^+$ gate were collected per sample. Flow cytometry data were analyzed using FlowJo software (TreeStar).

T cell receptor excision circle (TREC) measurement. TREC measurement was performed by real-time polymerase chain reaction (PCR) as described elsewhere [12]. Bead-separated $CD4^+$ and $CD4^-$ T cell subsets were used for determination of TREC levels. A value of TREC per microliter of blood was calculated on the basis of the value of TREC per 1×10^6 T cells and the absolute $CD4^+$ and $CD8^+$ T cell counts at the same time point.

Statistical analysis. Median values were used for all analyses, and median values with interquartile ranges (IQRs) are reported. The significance of paired differences (within each group) between weeks 0 and 24 was determined by the sign test. Unpaired analyses (between the 2 groups) were performed by the Wilcoxon rank-sum test. The association between variables was determined by Spearman's rank correlation. Because

of the exploratory nature of the present study, there was no adjustment for multiple testing, and calculated P values are reported.

RESULTS

Patient characteristics and CD4⁺ T cell responses to IL-2. There were no statistically significant differences between NRs and control subjects by the 2-sample Wilcoxon rank-sum test (table 1). The viral load of the NR group was higher than that of the control group (4862 vs. 728 copies/mL; $P = 0.1$) (table 1). When the between-group comparison was performed as a paired test, NRs had a statistically higher HIV load than did the control subjects ($P = .02$, sign test). The a priori decision had been to perform an independent analysis to achieve more robust statistical significance, particularly in view of the small size of the cohort, the lack of correction for multiple comparisons, and the lack of a complete set for certain parameters tested. As anticipated on the basis of the definition of NRs and control subjects, a significant difference was evident between the 2 groups in the CD4⁺ T cell count change from baseline at week 24 (−1.3% vs. 116.5%; $P \leq .0001$) (table 1). No statistically significant changes in the viral load were noted in either group throughout the duration of the study (data not shown).

Proportions of naive and memory T cell subsets at baseline. The proportion of CD4⁺ T cells with naive (19.1% [IQR, 9.1%–29.2%] vs. 21.9% [IQR, 16.9%–28.9%]), central memory (55.6% [IQR, 43.3%–67.0%] vs. 59.6% [IQR, 52.1%–63.3%]), effector memory (17.2% [IQR, 13.6%–30.8%] vs. 15.4% [IQR, 7.3%–28.4%]), and effector (0.2% [IQR, 0.1%–0.5%] vs. 0.3% [IQR, 0.0%–2.2%]) phenotypes at week 0 did not differ significantly between the NR and control groups ($P > .5$, for all) (figure 1*A*). Similarly, the proportion of CD8⁺ T cells with naive (8.6% [IQR, 4.2%–16.9%] vs. 16.5% [IQR, 13.9%–22.4%]; $P = .06$), central memory (51.3% [IQR, 38.8%–56.3%] vs. 45.1% [IQR, 35.6%–53.7%]; $P = .5$), effector memory (21.7% [IQR, 15.7%–35.5%] vs. 20.8% [IQR, 18.0%–27.3%]; $P = .7$) and effector (14.8% [IQR, 8.6%–19.9%] vs. 13.8% [IQR, 9.1%–18.6%]; $P = 1.0$) phenotype did not differ significantly at baseline between NRs and control subjects (figure 1*B*), with the exception of a trend toward a lower proportion of naive CD8⁺ T cells in the NR group.

TREC levels and T cell proliferation in CD4⁺ and CD8⁺ T cells at baseline. The TREC content in CD4⁺ and CD8⁺ T cells was measured at weeks 0 and 24 in both groups. At baseline, NRs had significantly lower TREC content than did control subjects in both CD4⁺ (15,700 [IQR, 6800–29,000] vs. 33,400 [IQR, 23,200–50,700] copies/1×10^6 T cells; $P = .03$) and CD8⁺ T cells (5200 [IQR, 1900–17,700] vs. 17,900 [IQR, 10,200–37,200] copies/1×10^6 T cells; $P = .03$) (figure 2*A*). Similar findings were observed when TREC levels were measured per microliter of blood (in CD4⁺ T cells, 4.0 [IQR, 3.4–17.0] vs. 15.9 [IQR, 8.5–24.7] copies/μL; $P = .05$) (in CD8⁺ T cells, 8.1 [IQR, 3.5–16.8] vs. 24.5 [IQR, 8.6–42.3] copies/μL; $P = .03$).

T cell proliferation was measured by intracellular Ki67. Consistent with the TREC data, NRs had higher levels of Ki67 expression than did control subjects in both CD4⁺ (9.9% [IQR, 6.6%–11.7%] vs. 5.8% [IQR, 4.9%–6.8%]; $P = .03$) and CD8⁺ (9.2% [IQR, 3.3%–12.3%] vs. 3.9% [IQR, 2.8%–4.6%]; $P = .03$) T cells (figure 2*B*). Similar findings were observed when activation of T cells was measured by surface CD38 expression (figure 2*C*). Expression of PD-1, a marker associated with activation and exhaustion of T cells [13], was higher on CD4⁺ T cells from NRs than on those from control subjects at baseline (16.4% [IQR, 8.5%–20.5%] vs. 9.3% [IQR, 7.8%–11.6%]; $P = .06$). Similarly, coexpression of HLA-DR with CD38 on CD4⁺ T cells, another marker of T cell activation, was higher for NRs than for control subjects at baseline (27.5% [IQR, 16.8%–34.9%] vs. 19.9% [IQR, 16.8%–27.7%]). Study of CD25 (IL-2 receptor α chain), CD127 (IL-7 receptor α chain), HLA-DR, Bcl-2, and intracellular cytotoxic T lymphocyte–associated protein 4 expression in CD4⁺ and CD8⁺ T cells, as well as PD-1 or HLA-DR/CD38 coexpression on CD8⁺ T cells, showed no

Table 1. Participant characteristics.

Characteristic	NRs ($n = 15$)	Control subjects ($n = 15$)
Age, years	41 (38–49)	38 (29–44)
CD4⁺ T cell count, cells/μL	416 (329–531)	403 (351–511)
CD8⁺ T cell count, cells/μL	1159 (819–1766)	1064 (721–1487)
CD4:CD8 ratio	0.32 (0.22–0.60)	0.37 (0.28–0.46)
Nadir CD4⁺ T cell count, cells/μL	242 (190–390)	251 (165–347)
HIV RNA load, copies/mL	4862 (499–30,000)	728 (54–3000)
Proportion receiving HAART[a]	7/15	8/15
Percentage change in CD4⁺ T cell count at week 24	−1.3 (−7 to 7)	116.5 (81 to 143)

NOTE. Data are median (interquartile range) values, unless otherwise indicated. HAART, highly active antiretroviral therapy; NRs, nonresponders.

[a] The remaining participants were receiving single or dual nucleoside analogues (1992–1996).

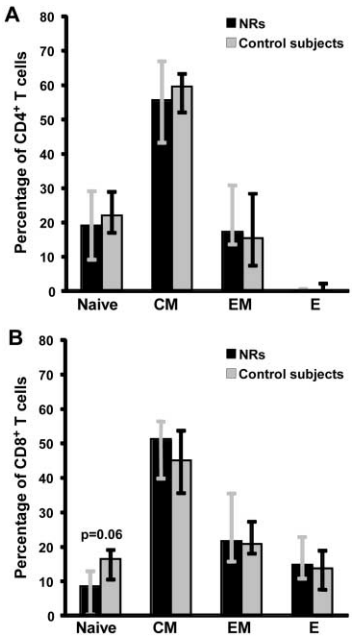

Figure 1. Similar proportions of naive and memory cells in CD4⁺ and CD8⁺ T cell pools in nonresponders (NRs) and control subjects at baseline (week 0). The percentage of CD4⁺ *(A)* and CD8⁺ *(B)* T cells with a naive or memory phenotype was evaluated by CD45RO and CD27 staining. No statistically significant differences were noted between NRs and control subjects in the naive/memory subset composition of the T cell pools, with the exception of a trend toward a lower percentage of naive CD8⁺ T cells in NRs than in control subjects ($P = .06$). Bars represent interquartile ranges. CM, central memory; E, effector; EM, effector memory.

statistically significant differences between the 2 groups at baseline (data not shown).

Expression of FoxP3, a marker of regulatory T (T_{reg}) cells [14], in CD4⁺ T cells did not differ at baseline between NRs and control subjects (8.2% [IQR, 5.3%–10.4%] vs. 7.1% [IQR, 6.0%–9.6%]; $P = .9$). The percentage of CD4⁺ T cells with a central memory phenotype expressing FoxP3, which is typically the phenotype of conventional functional memory T_{reg} cells [15–17], was significantly higher in NRs than in control subjects (17.7% [IQR, 15.4%–23.6%] vs. 12.6% [IQR, 7.6%–13.5%]; $P = .004$) (figure 2*D*).

Expression of CD25 on CD4⁺ T cells after IL-2 administration. Increases in CD25 expression on CD4⁺ T cells is known to occur after IL-2 immunotherapy, and previous studies have shown a correlation between increases in CD25 expression and CD4⁺ T cell expansions [4, 11]. We sought to determine whether changes in CD25 expression on CD4⁺ T cells after IL-2 administration differed between the 2 groups. As shown in table 2, the percentage of CD4⁺ T cells expressing CD25 increased by 11.8% ($P < .001$) in NRs and by 28.5% in control subjects ($P < .001$) from week 0 to 24 (table 2). The increases in NRs were significantly smaller than those in control subjects ($P = .001$).

Expression of FoxP3 in CD4⁺ T cells with a central memory phenotype did not change after IL-2 immunotherapy in either group. There was a significant increase from baseline in the percentage of naive CD4⁺ T cells expressing FoxP3 in both groups, but this increase was significantly smaller in NRs than in control subjects (22.9% [IQR, 8.6%–26.6%] vs. 39.6% [IQR, 25.8%–51.1%]; $P = .008$) (figure 3).

Immune activation and TREC levels per 1×10^6 T cells after IL-2 immunotherapy. T cell proliferation (as measured by expression of Ki67) and activation (as measured by expression of CD38) decreased on T cells after IL-2 administration (week 24) in both groups (table 2). The TREC level per 1×10^6 T cells decreased significantly at week 24 in CD4⁺ T cells of control subjects ($-13{,}100$ copies/1×10^6 T cells; $P = .002$) and to a much lesser degree in CD8⁺ T cells of both control subjects (-3400 copies/1×10^6 T cells; $P = .06$) and NRs (-1700 copies/1×10^6 T cells; $P = .04$). No significant change was observed in the CD4⁺ T cells of the NR group (-1600 copies/1×10^6 T cells; $P = .6$) (figure 4*A* and table 2). In contrast, the TREC level per microliter of blood did not change significantly in control subjects in either CD4⁺ or CD8⁺ T cells. In

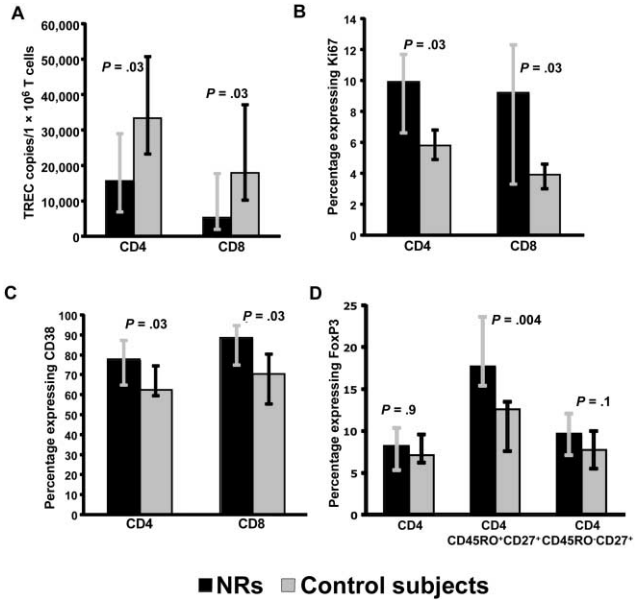

Figure 2. Lower T cell receptor excision circle (TREC) content, higher T cell proliferation, and higher forkhead box protein P3 (FoxP3) levels in central memory CD4⁺ T cells in nonresponders (NRs) than in control subjects at baseline (week 0). TREC level per 1×10^6 T cells was measured at week 0 and was found to be lower in NRs than in control subjects in both the CD4⁺ and the CD8⁺ T cell subset ($P = .03$, for both) *(A)*. T cell proliferation was higher in NRs than in control subjects in both CD4⁺ and CD8⁺ T cells ($P = .03$) *(B)*. CD38 expression was higher in NRs than in control subjects in both CD4⁺ and CD8⁺ T cells ($P = .03$) *(C)*. FoxP3 expression in central memory CD4⁺ T cells was higher in NRs ($n = 11$) than in control subjects ($n = 13$) ($P = .004$) *(D)*. Bars represent interquartile ranges.

Table 2. Paired changes from baseline (week 0) to week 24 after interleukin-2 administration.

Category, parameter	NRs (n = 15)	P (paired)	Control subjects (n = 15)	P (paired)	P[a]
$CD4^+$ T cells					
Change in count, cells/μL	−6 (−37 to 20)	1.0	419 (277 to 786)	<.001	<.001
Change in percentage of $CD4^+$ T cells expressing					
CD25	11.8 (9.2 to 19.2)	<.001	28.5 (20 to 35.8)	<.001	.001
Ki67	−2.1 (−4.0 to 0.0)	.06	−2.9 (−4.3 to −1.4)	.001	.6
CD38	−7.1 (−10.9 to −3.9)	<.001	−14.5 (−20.3 to −10.8)	<.001	.01
PD-1	−2.4 (−9.5 to −0.2)[b]	.07	−4.5 (−5.5 to −2.2)[c]	.003	.7
HLA-DR/CD38	−4.9 (−7.9 to −3.7)	.001	−7.8 (12.2 to −3.2)	<.001	.5
Change in TREC content					
Copies/1 × 10^6 $CD4^+$ T cells	−1600 (−5900 to 900)	.6	−13100 (−24600 to −8600)	.002	.002
Copies/μL of blood	−0.3 (−2.8 to 0.6)	.6	2.5 (−1.8 to 12.6)	.4	.1
$CD8^+$ T cells					
Change in counts, cells/μL	−221 (−595 to −108)	.04	219 (−44 to 374)	.2	.003
Change in percentage of $CD8^+$ T cells expressing					
CD25	0.6 (0.2 to 1.6)	<.001	2.7 (1.5 to 5.1)	<.001	.02
Ki67	−0.5 (−3.5 to 2.7)	.3	−0.4 (−1.3 to 0)	.04	.8
CD38	−0.9 (−4.2 to 0.9)	.6	−5.8 (−10.9 to 0)	.01	.2
PD-1	−1.1 (−11.1 to 1.8)[b]	1.0	−4.6 (−8.4 to 2.1)[c]	.6	.95
HLA-DR/CD38	−3.5 (−9.5 to 1.0)	.3	−6.7 (−12.7 to 2.3)	.2	.7
Change in TREC content					
Copies/1 × 10^6 $CD8^+$ T cells	−1700 (−6600 to −6)	.01	−3400 (−7400 to −50)	.005	.5
Copies/μL of blood	−4.7 (−8.9 to −0.4)	.002	−1.2 (−7 to 1.5)	.3	.3

NOTE. Data are median (interquartile range) values, unless otherwise indicated. NRs, nonresponders; PD, programmed death; TREC, T cell receptor excision circle.

[a] P value from Wilcoxon rank-sum comparison of the changes in NRs vs. control subjects.

[b] n = 11.

[c] n = 13.

NRs, there was a decrease in TREC level per microliter in $CD8^+$ T cells observed at week 24 relative to baseline (P = .007) (figure 4*B*).

DISCUSSION

Intermittent IL-2 immunotherapy in patients with HIV infection leads to $CD4^+$ T cell expansions that are associated with increases in $CD4^+CD25^+$ T cell levels and decreases in T cell proliferation and activation [5, 11]. In the present study, it was shown that increased baseline immune activation impeded $CD4^+$ T cell expansions induced by IL-2. It was also shown that, despite lack of increases in $CD4^+$ T cell numbers in the NR group, there was clear evidence of a biological effect of IL-2, as shown by the increase in CD25 expression on $CD4^+$ T cells.

Multiple studies have shown that IL-2 can lead to a significant, selective, and sustained $CD4^+$ T cell expansion. Recent data showed that newly emerging $CD4^+$ T cells are the product of peripheral expansion and do not represent recent thymic emigrants, are polyclonal with some intermediate features between naive and memory cells, and have elevated levels of FoxP3 expression with weak to moderate suppressive potential [5]. Ex vivo and in vivo labeling studies have confirmed that, although intense proliferation occurs during IL-2 administration, the $CD4^+$ T cell expansions are supported long term by decreased turnover and improved survival of naive and central memory $CD4^+$ T cells [7]. Clinical studies have also shown that some patients fail to experience $CD4^+$ T cell expansions after IL-2 immunotherapy or do so only after additional cycles beyond the 3–6 that are most typically used during the conventional duration of clinical trials.

In the present study, patients who had poor $CD4^+$ T cell responses to IL-2 were studied and matched by baseline $CD4^+$ T cell count to control subjects, who were patients participating in the same study who had good $CD4^+$ T cell responses to IL-2. A significantly higher level of immune activation and T cell proliferation in both $CD4^+$ and $CD8^+$ T cell pools were noted in NRs compared with control subjects. In agreement with this observation, significantly lower TREC content was observed in $CD4^+$ and $CD8^+$ T cells of poor responders compared with control subjects. Despite the above findings, the proportion of T cells with a naive phenotype did not differ at baseline between the 2 groups, suggesting that the activation state of the $CD4^+$ T cell pool may not be adequately addressed by its memory/

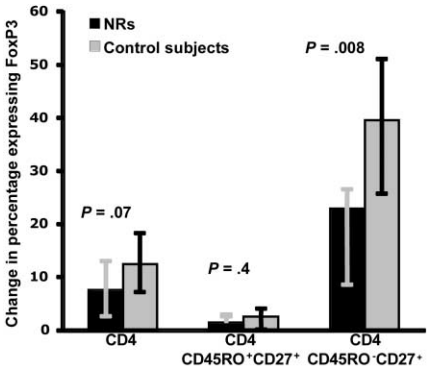

Figure 3. Increase in forkhead box protein P3 (FoxP3) expression in $CD4^+$ T cells of naive phenotype in both nonresponders (NRs) and control subjects but significantly greater change from baseline in control subjects. Levels of FoxP3 expression were measured in total, central memory, and naive $CD4^+$ T cells of NRs ($n = 11$) and control subjects ($n = 13$) before and after interleukin-2 administration. Increases were seen in the naive compartment only and were greater in control subjects than in NRs ($P = .004$).

naive subset composition. It is unclear whether these differences, in the presence of similar peripheral $CD4^+$ T cell count, represent longer infection times or interindividual variability with respect to immune activation responses or set point.

Regarding the proportion of $CD4^+$ T cells bearing a T_{reg} phenotype, a higher proportion of T_{reg} cells was noted in the central memory pool at baseline in those patients who had evidence of higher immune activation and did not have $CD4^+$ T cell responses after IL-2 administration, suggesting a compensatory mechanism. It is known that functional T_{reg} cells have a central memory phenotype, whereas $CD4^+$ T cells with a naive phenotype that express FoxP3 and/or CD25 may represent precursor of fully functional T_{reg} cells and may have weaker T_{reg} cell function. Cell availability precluded functional assays in our study. Future functional studies will be necessary to clearly delineate the role played by these different phenotypes in HIV-induced immune activation and their potential contribution to the expansions induced by IL-2 or other immune-based therapies. The clinical implications of these findings are important for new clinical trial designs addressing the potential role played by IL-2 in patients with HIV viremia either receiving or not receiving antiretrovirals. It is known that immune activation is directly associated with viral load [18, 19]. Thus, one would predict that HIV viremia, after treatment interruption or in patients with inadequately controlled viral load, would likely hinder $CD4^+$ T cell responses to IL-2. In fact, in a study of antiretroviral interruption after an IL-2 cycle, it was shown that the $CD4^+$ T cell responses during a viremic IL-2 cycle were blunted, compared with those observed in the same individuals receiving ART [20]. Similarly, the $CD4^+$ T cell responses of patients receiving IL-2 without antiretrovirals appear blunted compared with those achieved under HAART [21]. For patients already receiving IL-2, optimization of ART should be attempted if adequate IL-2 responses have not been observed.

Interestingly, in the present study IL-2 itself decreased immune activation and T cell proliferation and increased FoxP3 expression in phenotypically naive $CD4^+$ T cells even when $CD4^+$ T cell counts did not increase. This may explain why some patients eventually respond to IL-2 after additional cycles and even suggests the possibility that a potential $CD4^+$ T cell loss during that period was actually averted. That TREC content in $CD4^+$ T cells did not change in people who did not experience $CD4^+$ T cell expansions could be explained by decreased T cell proliferation relative to that in control subjects or preferential survival of TREC-enriched T cell subsets. Labeling studies have clearly shown that intense T cell proliferation is seen with IL-2 regardless of the long-term effect on $CD4^+$ T cell expansion, making the latter explanation more plausible [7]. In agreement also with in vivo labeling studies [7], a dilution of TRECs in $CD8^+$ T cells was observed, suggesting that, despite $CD8^+$ T cell proliferation, there is no effect on $CD8^+$ T cell survival and thus no $CD8^+$ T cell expansion with IL-2.

Finally, the present data are also consistent with the notion that increased immune activation in the T cell pool can in-

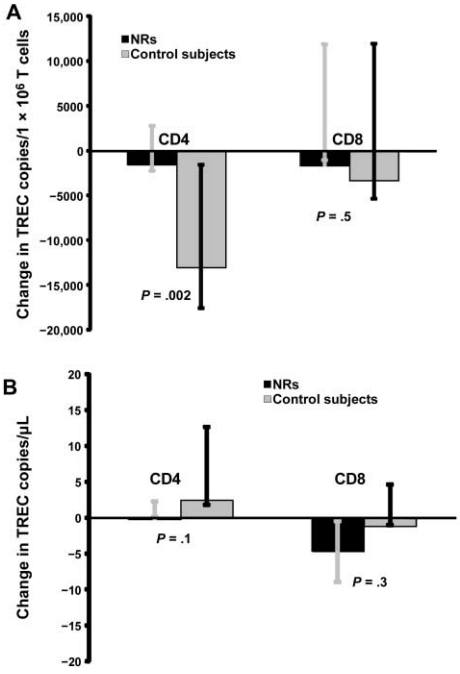

Figure 4. No significant difference in changes in T cell receptor excision circle (TREC) levels per microliter of blood from week 0 to 24 between nonresponders (NRs) and control subjects. TREC levels per 1×10^6 T cells decreased significantly in $CD4^+$ T cells of control subjects, with smaller decreases observed in $CD8^+$ T cells of both NRs and control subjects *(A)*. Changes in total TREC content per microliter of blood were compared between NRs and control subjects and were not significantly different despite a lack of $CD4^+$ T cell expansion in NRs at week 24 *(B)*.

dependently affect disease prognosis and response to therapy in HIV infection, as has been shown previously [22–25]. It is known that immune activation can have deleterious effects on $CD4^+$ T cell function even when $CD4^+$ T cell numbers are restored with HAART [26]. In the present study, increased activation of T cells was documented by several different markers, including Ki67, CD38, and PD-1. Increased FoxP3 expression on $CD4^+$ T cells with a central memory phenotype was also observed in NRs, suggesting the possibility of a compensatory mechanism. Although activated T cells tend to express lower levels of IL-7 receptors—such that their survival may be affected, hampering their peripheral expansion potential [27, 28]—in the present study a relationship between expression of the IL-7 receptor and IL-7 levels was not seen. It is feasible that CD127 down-regulation is a late event, occurring after loss of cells with a naive phenotype; in our cohort, the proportion of naive T cells was preserved.

In summary, we have shown that immune activation can adversely affect IL-2–induced $CD4^+$ T cell expansions. Even in the absence of $CD4^+$ T cell increases, IL-2 seems to induce biological changes in the T cell pool, including increases in $CD4^+CD25^+$ T cell levels and decreases in immune activation and proliferation. These findings underscore the limitations of the T cell pool to peripherally expand in settings of persistent immune activation and the potential influence of HIV viremia in successes and failures of immune-based therapies.

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